

Large Area (Mosaic) C₆₀⁺ IMAGING BY TOF-SIMS

OVERVIEW

Imaging of biological specimens is an area of growing interest in time-of-flight SIMS (TOF-SIMS). The difficulties of such analyses include preparation of the biological specimens for analysis in a vacuum environment without disturbing the distribution of the analyte of interest. An equally pressing concern is the efficient desorption and detection of high mass molecules that are of analytical importance. These analytical difficulties are exacerbated by adsorbed and adventitious overlayers such as water, short chain hydrocarbons and silicone residues. Moreover, tissue cross-sections are often quite large with respect to the typical raster size of an analytical ion probe. Analysis of

such large specimens requires the sample to be moved under the analytical ion probe in order to analyze the entire surface of interest.

In this Note we demonstrate mosaic imaging of a freeze-dried rat liver cross-section. The liver was exposed *in vivo* to a pharmaceutical agent. To generate the large area image of the liver cross-section, the sample stage is rastered under the analytical ion probe. Each tile of the large area image is "stitched" together to generate a single mosaic image. Each tile of the mosaic image retains its inherent pixel density.

The liver cross-section was analyzed in the as-received state; no treatment was performed on the specimen prior to analysis. Consequently, adsorbed surface layers of water, hydrocarbons

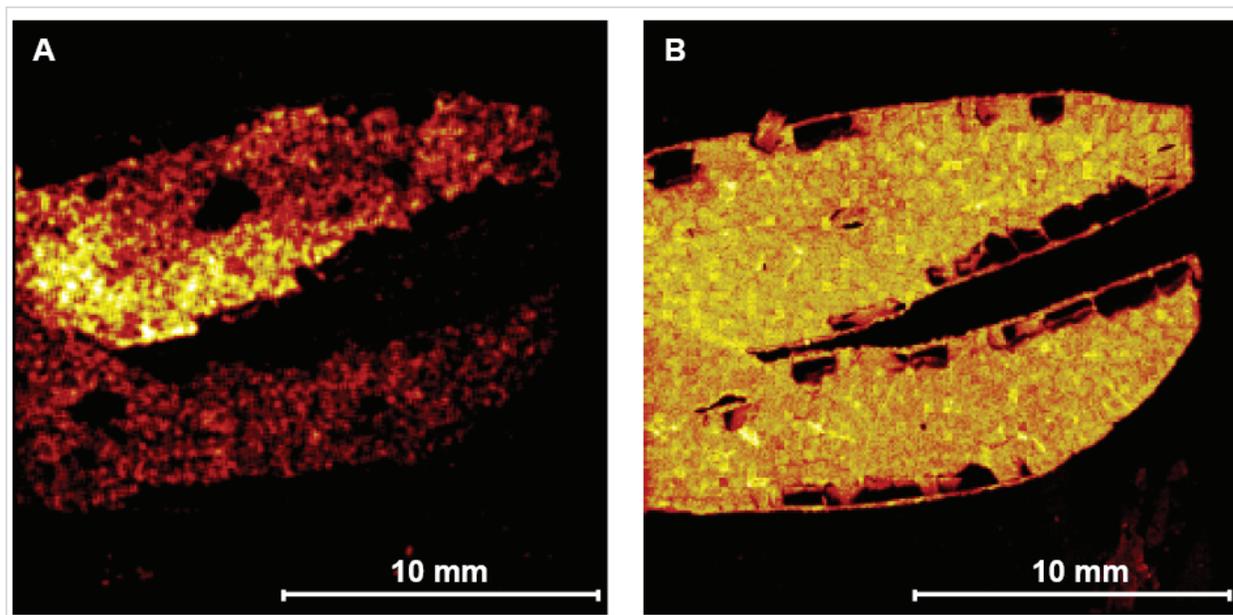


Figure 1: Mosaic images (19 mm x 19 mm total area) of a liver cross-section showing the lateral distributions of (A) drug and (B) phospholipids.

and silicones masked the underlying drug signals during analysis using a liquid metal ion gun (LMIG: Au⁺, Au₃⁺). Therefore, a C₆₀⁺ ion probe was employed for analysis.

EXPERIMENTAL

A 20 keV C₆₀⁺ ion beam was used to acquire the positive polarity (+SIMS) raw data stream mosaic images. The acquisition parameters included 148.4 μm field-of-view per tile at 4 seconds of data collection per tile, and a total stage raster area of 19 mm. The mosaic image was comprised of 128 x 128 tiles each having a 256 x 256 pixel density. The total acquisition time was approximately 7.5 hours (450 minutes). Analysis was performed with the sample at room temperature, and no charge compensation was required during analysis.

RESULTS

The nominal mass-to-charge (m/z) ratio of the drug molecular ion, detected as [M+H]⁺, is greater than 300 m/z . Using LMIG primary ions (i.e. Au⁺, Au₃⁺), the sensitivity toward the molecular ion of the drug was insufficient for imaging; therefore, C₆₀⁺ primary ions were used for analysis. Mosaic

images of the liver cross-section are presented in Figure 1. Figure 1A indicates the lateral distribution of the drug while Figure 1B indicates the lateral distribution of phospholipids. The phospholipid image was generated using the mass fragment at 184 m/z and indicates the general structure of the liver cross-section. The drug is present throughout the liver cross-section, but appears more concentrated in one lobe of the liver.

CONCLUSION

TOF-SIMS is useful for determining the lateral distribution of bioactive agents in prepared biological specimens. Modern ion source technology has enabled such analyses through the efficient desorption and ionization of high mass molecular species. Additionally, imaging of large specimens within a reasonable time period is a viable option owing to advanced data handling and the increased ion yields afforded by a C₆₀ primary ion source. This analytical capability has been demonstrated by imaging the distribution of a drug in a freeze-dried liver cross-section.